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Letters to the Editor

Dear Editor

Re: Uesugi M, Jasin HE. Macromolecular transport across the superficial layer of articular cartilage. Osteoarthritis Cart 2000;8:13–6.

In the paper by Uesugi and Jasin,[1] the authors studied the effect of the surface layer of articular cartilage on solute transport using several macromolecular solutes (serum albumin, IgG, ferritin). They concluded that 'the outermost surface layer does not constitute a barrier to the transport of macromolecules into the deeper zones of the tissue'. I believe their conclusion is incorrect due to an error in the design of their transport experiments.

The authors state that their transport studies 'were performed as described by Maroudas'.[2] However, they modified the technique in such a way as to result in significant artifacts. Briefly, intact or surface removed whole carpal bones were immersed in radiolabeled solutes for 4 h, after which full-thickness slices of cartilage were removed from the bone and the labeled solutes sequentially desorbed. Desorption plots (solute desorption vs time) were used to compare the transport of the different solutes for the intact and surface removed cartilage. There are two major errors in the authors experimental design. First, solute desorption out of the removed cartilage is not the same as absorption into the cartilage-bone. Transport in the former will occur through six surfaces (three-dimensional), only one of which is through the articular surface, while the later is essentially one-dimensional transport into the cartilage matrix through the surface. Thus, any effect of the surface layer on restricting solute transport would probably be masked by the flux of solute out of the matrix through the cut surfaces. Second, while desorption measurements can be used to determine the rate at which the solute is transported out of the entire matrix (mass transport), the method requires that the solute be equilibrated within the cartilage matrix before desorption begins, which for the macromolecular solutes used usually requires at least 48 h. If sufficient time is not allowed for solute equilibration, the solute will be non-homogeneously distributed within the matrix, with the greatest concentration at the articular surface. Furthermore, since the equilibrium partition of these solutes will depend on the glycosaminoglycan (GAG) content within the cartilage matrix, the spatial distribution of the solute will again be non-homogeneously distributed throughout the matrix thickness. In either case, knowledge of the solute's spatial distribution within the cartilage matrix would have to be known *a priori* in order to determine the rate of transport out of the matrix using desorption techniques.[3] Both these initial and boundary conditions were clearly discussed by Maroudas.[2]

Therefore, while the authors did transport the solutes into the cartilage matrix through the articular surface (intact and removed), they did not measure the transport through the surface into the matrix but rather measured the transport out of the matrix through the surface and the cut edges (four sides and bottom). On the other hand, if the authors had waited a sufficient time for the solutes to reach equilibrium within the matrix and then desorbed the whole bone, performed first with an intact surface and then repeated after surface removal, they could have determined the affect of the surface layer from either the total amount of absorbed solute or the desorption vs. time profiles. As found in my own experimental studies, the surface layer does effect transport of macromolecular solutes,[4] as do the GAGs themselves.[5]

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Dear Editor

Re: Uesugi M, Jasin HE. Macromolecular transport across the superficial layer of articular cartilage. Osteoarthritis Cart 2000;8:13–6. Response to letter from Dr P. A. Torzilli.

We thank Dr Torzilli for his comments on our recent paper.[1] Although his arguments are cogent, we do not think that they are pertinent to our work. In the first place, our conclusions were based on a comparison of molecular exchange rates in intact cartilage explants and in explants treated with elastase before slicing them off the bone. Torzilli[2] sliced the superficial 50–100 μm of the cartilage,

thus disrupting the collagen fiber mesh at the surface and also discarding the subsurface area which is almost devoid of acidic aggrecan. The two methods are quite different and do not lend themselves to a meaningful comparison. It is obvious that the operational definitions of surface layer in our work and that of Torzilli are completely different.

By comparing similar samples with and without enzyme digestion, we made the cut edges identical between control and experimental samples, and the molecular exchange taking place at the level during the incubation step should also be identical between samples. Moreover, Dr Torzilli failed to take into account the fact that the explants were incubated with solutions containing the unlabeled proteins with the same concentrations than the solutions used in the initial incubation step. Thus, we are not dealing with a gradient-driven diffusion process, but only with a rate of exchange between labeled proteins within the tissue and unlabeled proteins outside the tissue. During the final incubation step, there would still be slow diffusion of the different macromolecules into the tissue. It was for this reason that we limited the first incubation step to 4 h. We wanted to make sure that the labeled proteins would not elute through the cut surface; a process that would almost surely occur had we incubated the bones to equilibrium.

In summary, our experiments were designed in such a way that only a small amount of the labeled macromolecules needed to accumulate just under the articular surface, an area almost devoid of aggrecan. Our conclusions were based on a strict comparison of data between intact surfaces and surfaces digested with elastase. In a previous publication,[3] we had shown that such treatment resulted in unmasking of the subjacent collagen without detectable depletion of the acidic proteoglycans within the matrix. The apparent discrepancy between their work and ours as to the role of the surface layer can be ascribed to the different methodology employed.

We thank Dr Torzilli for his interest in our work.

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